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(54) Title: AGENTS FOR THE PREVENTION AND TREATMENT OF BREAST CANCER

(57) Abstract

Agents and methods for the diagnosis and therapy of breast cancer and related conditions are disclosed. Such agents include antisense molecules of three genes implicated in breast cancer and promoter elements which control the transcription of these genes, as well as analogues and derivatives, and protein molecules expressed by these genes.

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TITLE OF THE INVENTION:

AGENTS FOR THE PREVENTION AND TREATMENT OF BREAST CANCER

FIELD OF THE INVENTION:

The invention relates to therapeutic agents for the prevention and treatment of breast cancer, especially in humans. More specifically, the invention relates to two genes, BC534 and BC538, that are implicated in causing breast cancer, to antagonists of these genes and their gene products. The invention additionally relates to nucleic acid molecules that influence the expression of these genes. The invention also relates to therapeutic methods that employ all such agents.

BACKGROUND OF THE INVENTION:

I. Breast Cancer

As an inherited trait breast cancer is one of the most common genetic diseases in the industrial world; in fact, one out of 100 women alive today will develop breast cancer due to inheritance unless a cure for the disease is found. About 40% of cases are diagnosed before the patient has attained the age of 30. Only about 5% of all breast cancer is, however, inherited. The remaining 95% of breast cancers result from the acquisition of mutations.

Breast cancer is presently treated using surgery, endocrine therapy and chemotherapy (Salmon, S.E., Semin. Oncol., 17:50-52 (1990); Hortobagyi, G.N., Breast Cancer

Res. Treat 21:3-13 (1992). Endocrine therapy results in complete or partial remissions in only 30% of patients (Jiang, S.-Y., et al., J. Natl. Canc. Inst. 84:580-591 (1992); Muss, H.B., Breast Cancer Res. Treat 21:15-26 (1992)). Chemotherapy, despite the development of new antineoplastic agents, has had only limited success in treating breast cancer (Hortobagyi, G.N., Breast Cancer Res. Treat 21:3-13 (1992)). Thus, surgery is the only presently proven treatment for breast cancer; its use has long been controversial (Albert, S. et al., Cancer 41:2399-2408 (1978)). Surgery is often combined with endocrine therapy or chemotherapy regimes.

Biological agents, such as interferon and interleukin, have been found to be capable of producing definite anti-tumor responses. Unfortunately, such advances have not yet led to improved regimens for managing breast cancer (Hortobagyi, G.N., Breast Cancer Res. Treat 21:3-13 (1992)). Monoclonal antibodies have also been employed, however, the available antibodies have proven to lack sufficient sensitivity or specificity to selectively target tumor cells (Hortobagyi, G.N., Breast Cancer Res. Treat 21:3-13 (1992)).

II. Oncogenes and Tumor Suppressor Genes

One mechanism through which cancer may arise is through a cell's exposure to a carcinogenic agent, either chemical or radiation. Such exposure may damage the DNA sequence of critical genes present in the genome of a cell of an animal. If this damage leads to either an impairment in the expression of the gene, or in the production of a mutant gene product, the cell may then proceed to proliferate, and ultimately result in the formation of a tumor.

One class of such critical genes has been referred to as "oncogenes." Oncogenes are genes which are

naturally in an "inactivated" state, but which, through the effect of the DNA damage are converted to an "activated" state capable of inducing tumorigenesis (i.e. tumor formation). Oncogenes have been identified in 15-20% of human tumors. The products of oncogenes ("oncoproteins") can be divided into two broad classes according to their location in the cell.

Oncogene products which act in the cytoplasm of cells have readily identifiable biochemical or biological activities (Green, M.R., Cell 56:1-3 (1989)). Those that act in the nucleus of a cell have been more difficult to characterize. Some nuclear oncoproteins (such as E1A and myc) have transcriptional regulatory activity, and are believed to mediate their activities by the transcriptional activation of cellular genes (Kingston, R.E., Cell 41:3-5 (1985)). Other nuclear oncoproteins appear to have a complex array of activities (such as DNA binding activity, ability to initiate viral DNA synthesis, ATPase activity, helicase activity, and transcriptional regulatory activity) (Green, M.R., Cell 56:1-3 (1989)).

The creation of a mutant oncogene is only one of the requirements needed for tumor formation; tumorigenesis appears to also require the additional inactivation of a second class of critical genes: the "anti-oncogenes" or "tumor-suppressing genes." In their natural state these genes act to suppress cell proliferation. Damage to such genes leads to a loss of this suppression, and thereby results in tumorigenesis. Thus, the deregulation of cell growth may be mediated by either the activation of oncogenes or the inactivation of tumor-suppressing genes (Weinberg, R.A., Scientific Amer., Sept. 1988, pp 44-51).

Oncogenes and tumor-suppressing genes have a basic distinguishing feature. The oncogenes identified thus far have arisen only in somatic cells, and thus have been incapable of transmitting their effects to the germ

line of the host animal. In contrast, mutations in tumor-suppressing genes can be identified in germ line cells, and are thus transmissible to an animal's progeny.

The "p53" gene encodes a nuclear protein that forms a stable complex with both the SV40 large T antigen and the adenovirus E1B 55 kd protein. The p53 gene product may be inactivated by binding to these proteins.

Initially, the p53 gene was thought to be an oncogene since it is capable of immortalizing primary rodent cells and can cooperate with the ras oncogene to cause transformation. Subsequent research, however, has revealed that the p53 genes used in those early experiments was a mutant allele of the normal p53 gene (Green, M.R., Cell 56:1-3 (1989)), and that the p53 gene is a tumor-suppressing gene rather than an oncogene (Green, M.R., Cell 56:1-3 (1989); Mowat et al., Nature 314:633-636 (1985)).

Mutations at any of a large number of positions in the p53 gene can result in the activation of the transforming potential of the p53 gene product (Eliyahu et al., Nature 312:646-649 (1984); Finlay et al., Molec. Cell. Biol. 8:531-539 (1988)). This has suggested that the activation of the p53 transforming activity is due to the inactivation of the normal p53 activity (Green, M.R., Cell 56:1-3 (1989)).

The p53 gene has been implicated as having a role in colorectal carcinoma (Baker, S.J. et al., Science 244:217-221 (1989)). Studies had shown that allelic deletions of the short arm of chromosome 17 occurred in over 75% of colorectal carcinomas. The region deleted was subsequently found to encompass the p53 gene locus (Baker, S.J. et al., Science 244:217-221 (1989)). The deletion of the region was found to mark a transition from a (benign) adenocarcinoma stage to a (malignant) carcinomatous stage (Vogelstein, B. et al., New Engl. J. Med. 319:525 (1988)).

Similar deletions in chromosome 17 have recently been identified in a wide variety of cancers including breast and lung cancers (Mackay, J. et al., Lancet ii:1384 (1988); James, C.D. et al., Canc. Res. 48:5546 (1988); Yakota, J. et al., Proc. Natl. Acad. Sci. (U.S.A.) 84:9252 (1987); Toguchida et al., Canc. Res. 48:3939 (1988); Frebourg, T. et al., J. Clin. Invest. 90:1637-1641 (1992); Poller, D.N. et al., Brit. J. Canc. 66:583-588 (1992); Barbareschi, M. et al., Anatom. Pathol. 99:408-418 (1992); Sawan, A. et al., J. Pathol. 168:22-28 (1992)). In addition to p53 allele loss, Nigro et al. (Nature 342:705-708 (1989)) have demonstrated that the single remaining p53 allele in a variety of human tumors (brain, colon, breast, lung) undergo a point mutation which renders it tumorigenic. Fearon et al. (Cell 61:759-767 (1990)) have hypothesized that both point mutations and deletions in the p53 alleles may be required for a fully tumorigenic phenotype. These findings suggest that the p53 gene may have a role in many types of cancers.

The role of p53 in tumor formation had engendered a variety of methods for identifying and detecting p53 expression. Levine, A.J. et al., PCT appl. WO92/00311 discusses the use of a panel of probes to distinguish between normal and mutant p53 genes. Vogelstein, B. et al., European patent applications publication number 390,323 discusses a method for diagnosing the presence of mutation in the p53 gene. Clark, J.S. et al. (Canc. Lett. 66:193-200 (1992)) describe the use of dual flow cytometry to assess p53 expression in breast cancer tumor cells. The ability to generate antibodies that are reactive with the p53 protein has led to the development of immunoassays capable of discerning p53 expression in the sera of cancer patients (Hassapoglidou, S. et al., Clin. Biochem. 25:445-449 (1992); Reynolds, F.H. et al., PCT appl. publication number WO92/13970).

Vogelstein, B. et al., European patent application publication number 518,650 discusses a method of treating cancer involving the administration of p53 polypeptides. The use of p53 polypeptides in the treatment of cancer is also discussed by Lee, W.H. et al. in European patent appl. publication number 475,623. This document discloses the sequence of the p53 cDNA and the encoded protein, and is herein incorporated by reference.

Smith L.J. (U.S. Patent No. 5.087,617) discusses the use of an antisense oligonucleotide in the treatment of cancer, and in particular, the use of a p53 antisense oligonucleotide in a gene therapy approach to cancer treatment. The use of antisense technology in the treatment of breast cancer is also discussed by Kenney, N. et al. (Breast Canc. Res. Treat. 19:184 (1991)) and by Watson, P.H. et al., (J. Cell Biol. 115:440A (1991) and J. Canc. Res. 51:3996-4000 (1991)).

BRIEF DESCRIPTION OF THE FIGURE:

Figure 1 presents the cDNA and encoded amino acid sequences of BC534, BC538, BC538.1 and BC53/reg. Figure 1A shows the nucleotide sequence of BC534 cDNA. The sequence is 100% homologous to nucleotides 494-559 inclusive on the antisense strand of p53 mRNA (nucleotides 12,204-12269 inclusive on the antisense strand of p53 gene). Figure 1B shows the 2.35 kd, 21 amino acid nuclear protein encoded by the BC534 sequence. Figure 1C shows the nucleotide sequence of BC538 cDNA. The sequence is 100% homologous to nucleotides 82-216 inclusive on the antisense strand of p53 mRNA . It comprises two exons (seperated by an intron of 10,729 bases) linking nucleotide 11,467-11,496 inclusive plus nucleotide 631- -736 on the antisense strand of p53 gene. Figure 1D shows the 4.98 kd nuclear protein encoded by the BC538 sequence. Figure

1E shows the nucleotide sequence of BC538.1 . The sequence is 100% homologous to nucleotides 11,467-11,718 inclusive on the antisense strand of p53 gene. BC538.1 includes the first 30 nucleotides of BC538 and the first 222 nucleotides from the intron which seperates BC538 exon #1 and Exon#2. Figure 1F shows the 9.27kD protein encoded by BC538.1. Figure 1G shows the transcription regulatory region ("BC53/reg") of the BC53 family of genes. The regulatory region is 100 % homologous to the region of the antisense strand of p53 gene consisting of nucleotides 12,411, 12,518 inclusive. It contains a "CCAAT box" (nucleotide 12,570-12,581), two "TATA boxes" ("1" nucleotide 12,481-12,499 and "2" 12,411-12,570) and a cap site (nucleotide 12,463-12,470). The CCAAT box, one TATA box "1" and the cap site are correlated.

Figure 2A/B shows the organisation of the BC53 family of genes including location of "AATAA" transcription termination signals.

SUMMARY OF THE INVENTION:

The invention concerns agents and methods for the diagnosis and therapy of breast cancer and related conditions. Such agents include antisense molecules capable of influencing the transcription of either of the genes, BC534, BC538 and BC538.1. The invention also includes antagonists of the products of these genes.

In detail, the invention provides a nucleic acid molecule, substantially free of natural contaminants, that encodes a protein selected from the group consisting of BC534, BC538 and BC538.1.

The invention also provides a protein, substantially free of natural contaminants, selected from the group consisting of a BC534 gene product, a BC538 gene product and a BC538.1 gene product.

The invention also provides a reagent capable of diagnosing the presence of a molecule selected from the group consisting of a BC534 gene sequence, a BC534 mRNA transcript, a BC534 gene product, a BC538 gene sequence, a BC538 mRNA transcript, a BC538 gene product, a BC538 mRNA transcript and aBC538.1 gene product.

The invention particularly concerns the sub-embodiments wherein the reagent is a nucleic acid molecule (particularly a ribozyme produced from nucleic acid molecules having a sequence of BC534, BC538, BC538.1 or BC53/reg or a nucleic acid molecule obtainable by mutating a nucleic acid molecule having a sequence of BC534, BC538, BC538.1 or BC53/reg or which comprises a nucleic acid sequence that is complementary to the nucleotide sequence of BC534, BC538,BC538.1 or BC53/reg) or a protein (such as an antibody, or a fragment of an antibody, especially one which is capable of binding to a BC534, BC538 or BC538.1 gene product.

The invention also provides a method of treating breast cancer which comprises providing to an individual, in need of such treatment, an effective amount of an inhibitor of BC534, BC538, BC538.1 or BC53/reg especially wherein the inhibitor is a protein (such as an antibody, or fragment thereof) or a nucleic acid molecule.

DESCRIPTION OF THE PREFERRED EMBODIMENTS:

The phosphoprotein p53 localise to chromosome 17p13 was the first confirmed inherited human breast cancer antigen, and is the confirmed factor in about 1% of all inherited cancers (Malkin, D. et al., Science 250:1233-1238 (1990); Frebourg, T. et al., J. Clin. Invest. 90:1637-1641 (1992)). There is strong genetic evidence that there is another gene in the region of chromosome 17q21-23 shared possibly a tumor suppressor, which is a factor in the majority of inherited breast tumors. One

very strong possible is believed to be a 17ß hydroxy steroid dehydrogenase

The fact that mutations in p53 lead to breast cancer in a considerable number of females and is in fact found in germline mutations in a majority of women diagnosed with breast cancer under 30 years of age makes it a very important antigen in the pathophysiology of breast cancer. In addition, families carrying a mutated p53 gene have an extremely high rate of other cancers including brain tumors and sarcomas. There is, however, no proof that this gene plays a significant role in induced, i.e., non-inherited breast cancer. The mode of action of p53 in the pathophysiology of breast cancer is not known.

The present invention relates to the discovery of three genes, BC534, BC538 and BC538.1 which encode proteins with the potential to cause tumors in humans. The present invention provides the sequence of these molecules, as well as that of the proteins encoded by them. These molecules may be used in the diagnosis, prediction and treatment of breast cancer.

The BC534, BC538 and BC538.1 genes are antisense to regions of the human p53 gene, and thus their transcripts cannot be expressed under circumstances under which the p53 gene is normally expressed. The BC534, BC538 and BC538.1 genes thus cannot be expressed in healthy humans. The BC534, BC538 and BC538.1 genes can be expressed, however, if mutations or rearrangements occur in the p53 gene which impair or prevent the transcription of that gene. Silent mutations which might occur in the BC534, BC538 or BC538.1 genes will also influence the expression of the antisense protein. Pathogens and toxic substances to which the p53 gene might be exposed can also cause expression of BC534, Bc538 and BC538.1, but in these cases expression of the latter genes will stop once the

pathogen/toxic substance is removed from the intracellular environment.

I. The Molecules of the Present Invention

The negative strand of exons and introns in the human p53 gene was evaluated in a search for genes responsible for tumor formation. This evaluation led to the recognition that the p53 antisense strand encoded three proteins - designated herein as "BC534", "BC538" and "BC538.1", respectively.

A. The BC534 Protein

The BC534 cDNA sequence is shown in Figure 1A (SEQ ID NO:1) and the deduced sequence of the protein in Figure 1B (SEQ ID NO:2).

SEQ ID NO:1 CCCAGAATGCAACAAGCCCAGACGGAAACCGTAGCTGCCCT
GGTAGGTTTTCTGGGAAGGGACAGAAGATGACAGGGG

SEQ ID NO:2 MQEAQTETVAALVGFLGRDRR

The BC534 gene sequence (Figure 1A; SEQ ID NO:1) is 100% homologous to nucleotides 494-559 inclusive on the antisense strand of p53 mRNA.

The 2.35 kd, 21 amino acid biologically active peptide with a nuclear transport signal (nuclear protein) encoded by the antisense cDNA is shown in Figure 1B (SEQ ID NO:2). This protein can affect the intracellular regulation of the following human cancer-related genes/gene products: (1) met-proto oncogene, (2) oncogene related tyrosine kinase receptor Flt-gene which maintains normal conditions in lung, placenta, liver, kidney, heart and brain, (3) c-alb gene proto oncogene tyrosine kinase which is involved in chronic myelogranulous leukemia, (4) human neurofibromitosis

type 1 gene, (5) papilloma virus E1-1 protein, which is involved in the pathophysiology of genital cancers in humans, (6) estrogen sulfotransferase, which appears to control the level of estrogen receptor by sulfurylating estradiol; it has been demonstrated that mutations in the gene for the androgen receptor (a gene analogous to the estrogen receptor) which prevent proper interaction of androgen with the receptor can cause male breast cancer as well as androgen insufficiency, and (7) human lung surface active protein which is involved in neonatal infant deaths.

B. The BC538 Phosphoprotein

The BC538 gene sequence (Figure 1C; SEQ ID NO:3) is 100% homologous to nucleotides 82-216 inclusive on the antisense strand of p53 mRNA.

SEQ ID NO:3 TCCATGGACGAGAGGCGGAAGGCAGTCTGGCTGCCAATCCA
GGGAAGCGTGTCACCGTCGTGGAAAGCACGCTCCCAGCCCG
AACGCAAAGTGTCCCCGGAGCCCAGCAGCTACCTGCTCCCT
GGACGGTGGCTCTAGAC

The 4.98 kd phosphoprotein encoded by the antisense cDNA is shown in Figure 1D (SEQ ID NO:4).

SEQ ID NO:4 MAVTRKAVWLPIQGSVSPSWKARSQPERKVSPEPSSYLLPG
RWL

The protein has a protein kinase signature (GXGXXG), sites for cAMP and cGMP dependent phosphorylation, protein kinase c and casein kinase phosphorylation. The protein is a potential activator of (1) human basic conserved protein, (2) wnt-5b protein precursor, (3) int-2 proto oncogene protein precursor, (4) erbb-3 receptor protein tyrosine kinase, (5) zinc finger protein HF10, (6) rb protein, (7) galactosyl transferase

kinase, (8) acidic and (9) basic fibroblast growth factor receptor precursor which are involved in epithelial cell tumors, (10) a negative regulator of mitosis which inhibits cells from entering mitosis and prevents cells from leaving mitosis, (11) thyroid peroxidase, and (12) myelo peroxidase. All the above proteins, except myelo peroxidase, have been shown to be associated with, but not the cause of, the pathophysiology of female breast cancer.

C. The BC538.1 Phosphoprotein

The BC538.1 gene sequence (Figure 1E; SEQ ID NO:5) is 100% homologous to nucleotides 11467-11718 (this includes nucleotide 82-112 on the of p53 mRNA) on the antisense strand of p53 DNA.

SEQ ID NO:5 TCCATGGCAGTGACCCGGAAGGCAGTCTGGCTGCTGCAAG
AGGAAAAGTGGGGATCCAGCATGAGACGCTTCCAACCCTG
GGTCACCTGGGCCTGCAGAGGAGGAGGCCCCTCCGGGAAC
ACCATGCCAGTGTCTGAGACAGCTCGGCTCCCTGTGGTGC
AGGAAAAGAATGGCTGCTTCACATTCTCTCTTCCAATGTT
TCACCACAACCCAAGCACTCCTGCCCCACCCCACACCAGC
CATGCACTTCTTTGA

The 9.27 kd phosphoprotein encoded by the antisense cDNA is shown in Figure 1F (SEQ ID NO:6).

SEQ ID NO:6 MAVTRKAVWLLQEEKWGSSMRRFQPWVTWACRGGGPSGNT
MPVSETARLPVVQEKNGCFTFSLPMFHHNPPSTPAPPHTS
HALL

The protein has a protein kinase "GXGXXG" signature, and by similarities appears to have leukotriene-like properties. It can affect the regulation of a DNA polymerase, a toxic cysteine protease, thyroid

peroxidase, the activating and the endoprotease cleaving enzymes of the ubiquitin pathway, galactosyl transferase protein kinase, granulin/annexin (a disintegrin-like gene, localized at chromosome 17q/21.3, is somatically rearranged in some primary breast cancers), a member of the annexin family, and the human stem cell protein which is involved in t-cell leukemia and other hemopoetic malignencies.

D. The BC534/538/538.1 Transcription Regulation Sequences

The BC53 gene family 5'- regulatory sequences (Figure 1G; SEQ IC NO:7) are 100% homologous to nucleotides 12411-14581 inclusive on the antisense strand of the p53 gene.

SEQ ID NO:7 AAAAGCCAAGGAATACACGTGGATGAAGAAAAAGAAAAG
TTCTGCATCCCCAGGAGAGATGCTGAGGGTGTGATGGGA
TGGATAAAAGCCCAAATTCAAGGGGGGAATATTCAACTT
TGGGACAGGAGTCAGAGATCACACATTAAGTGGGTAAAC
TATAAAAAAACACTG

The 5'- regulatory region contains two distinct promoter systems P1 and P2. P1 is a correlated CCAAT/TATA/CAP promoter system and P2 is a TATA box. The dual promoter system indicates that BC53 family genes must be subject to different transcriptional activating signals as is the case with expression of the same gene in different type tissue.

II. The Physiological Role of the Molecules of the Present Invention

Under normal physiological conditions, the sense/antisense relationship between p53 and BC534/BC538/BC538.1 genes will not allow expression of

these genes in healthy humans; however, acquired mutations in regions of the p53 gene involved in the antisense repression of these genes will allow BC534/BC538/BC538.1 to be expressed, and thus result in tumor formation.

p53 has been described as a "policeman" against tumors, especially virus-induced tumors, in humans, and, at least in one case, has been demonstrated to bind to the offending viral protein; however, how the policing role is accomplished had not been previously determined. The antisense repression of several tumor associated proteins by p53 mRNA appears to be the method of control of the expression of cancer bearing proteins, and the range of tumor-related proteins repressed by p53 gene can explain the high incidence of other cancers found in families carrying a mutated p53 gene.

Nucleotide sequence comparisons done by us suggest that regions of the sense strand of chromosome 17q are partially duplicated on the antisense strand of chromosome 17p in the region encoding the p53 gene. Sequence duplication and predicted properties indicate that the three genes of the BC53 series either collectively or individually can cause inherited and early onset breast cancer in humans and in fact may be the primary cause of all the pathophysiological symptoms of the disease. The mechanism by which significant levels of BC534/BC538/BC538.1 are expressed in preference to or together with p53 is probably influenced by mutations, e.g. germline mutations in p53, and other specific cellular environmental factors. Significant amounts of BC534/BC538/BC538.1 cannot be expressed in normal humans because of the antisense repression by wild type p53 mRNA. The germline mutation at aa258 (Li-Fraumeni syndrome) which occurs in p53 and predisposes a small percentage of inherited breast cancer might activate an enhancer element which is required for initiation of transcription of the genes of

the BC53 family. In addition to the known mutation in chromosome 17p involving p53, which occurs in about 1% of inherited breast cancer, it has been demonstrated that mutations and deletions in the region of 17q/21-23 (the BRCA locus in the human genome for female breast cancer and ovarian cancer) account for the majority of early onset and inherited breast cancers.

With regards to the specific initiation mechanism which might involve gene products of the BC53 gene family it is likely that the gene products block the activity of an essential gene/protein located at 17q/21-23. In one example, proteins from the BC53 family genes block the activity of a gene/protein located at the BRCA locus ("brca"), in those cases where a simple mutation in the p53 gene is the primary cause. In the same way loss of activity of "brca", either by deletions or by mutations in the 17q/21-23 will also lead to early onset and "non-p53 related" inherited breast cancer.

Some of the genes, i.e. no. 2, 4, 6, 7, 11, 12 listed in: I.B. "The BC538 Phosphoprotein", occur in regions of chromosome 17q close to the "brca" region, and changes in activity of 2, 4, 6, 11 have been noted in many cases of female breast cancer. However, galactosyl transferase kinase (no 7; 17q/23), and myelo peroxidase (no 11; 17q/21.3) are located in more favorable positions. Over-expression of any one of these two proteins can be the trigger for initiation of cellular events resulting in breast cancer. Breast cancer related changes are common also in no 1, 3, 5, 8, 9 (see: I.B. "The BC538 Phosphoprotein"); these genes are located on chromosomes other than chromosome 17, in regions where mutations and deletions have been found in humans with breast cancer.

In a second example the leukotriene activity of BC538.1, which is normally blocked by "brca", is activated when mutations or deletions occur in "brca". This allows BC538.1 to influence activity of a critical

DNA polymerase, ubiquitin pathway enzymes (perphaps causing premature termination of critical cell cycle control proteins), a toxic thiol proteases, a galactosyl transferase kinase, and a thyroid peroxidase.

In a third example, mutations in p53 or mutations or deletions in the region of "brca" permit the expression of BC534 which leads to over-expression of estrogen sulfotransferase (chromosomal location not presently known), oncogene tyrosine kinase 'flt' and other tyrosine kinases which initiates events leading to breast cancer or ovarian cancer.

Breast and ovarian cancer in human females appear to have the same biochemical cause; therefore, the BC53 family genes are likely to be the cause of ovarian cancer. Ovarian tissue differs from breast tissue, and therefore factors influencing gene transcription in ovary are different from those in breast. Different promoters programming transcription of the same gene in different tissues appear to be the major choice in eukaryotes for regulating the expression of the same gene in different tissues. The two promoter systems 'BC53/reg' which can regulate expression of BC53 family genes are consistent with expression of BC534, BC538 and BC538.1 in both breast and ovarian tissue.

The genes and proteins discovered and described here, targets of normal p53 control, appear to be able to escape when programmed or incidental mutations occur in crucial regions of the p53 gene. The locus of these genes might be identified already as other breast cancer loci on chromosome #17; although any other localization is also feasible. The sense - antisense relationship between p53 and BC534/BC538 makes the latter proteins ideal candidates for the direction of immunotherapeutic reagents to treat and cure breast cancer in women. Also, because these proteins are not expressed in healthy women, side effects from any such therapeutics should be nonexistent.

Irrespective of the manner of the involvement of the BC534, BC538 or BC538.1 genes in breast cancer, any therapeutic method which blocks the expression of these proteins or blocks the proteins will significantly contribute towards the control of the disease. The diagnostic and/or therapeutic reagents of the invention additionally include fusion proteins, antipeptide reagents, etc., made from the deduced amino acid sequences described in the embodiment of Figures 1B and 1D. The invention also includes any cellular protein activated by the proteins or domains of the proteins described in the embodiment of Figures 1B, 1D or 1E.

III. The Uses of the Molecules of the Present Invention

The elucidation of the significance of BC534 or BC538 in the etiology of breast cancer provides improved means for diagnosing the presence and clinical grade of the disease. Moreover, it provides an improved means for predicting whether an asymptomatic individual is predisposed to the disease. It further provides a means for treating the disease.

In particular, any of the proteins described in Figures 1B, 1D or 1F or mutants thereof, may be used in the treatment of, or in the development of reagents for the treatment of, these diseases and conditions.

A. Diagnostic Uses

Since neither BC534, BC538 or BC538 are expressed (or expressed in significant amounts) by normal cells, the detection of these molecules in a tissue or fluid sample -- such as a biopsy sample, or a blood or lymph fluid sample -- is indicative of the presence of breast cancer in a patient.

The detection of these molecules may be done by any of a variety of methods. In one embodiment, antibodies

are employed that are capable of binding to the products of the BC534, BC538 or BC538.1 genes, and the presence of such molecules is determined via an immunoassay. A large number of suitable immunoassay formats have been described (Yolken, R.H., Rev. Infect. Dis. 4:35 (1982); Collins, W.P., In: Alternative Immunoassays, John Wiley & Sons, NY (1985); Ngo, T.T. et al., In: Enzyme Mediated Immunoassay, Plenum Press, NY (1985); incorporated by reference herein.

Suitable antibodies can be either polyclonal or monoclonal, of either a species homologous to or heterologous to the species from which the sample was derived. In lieu of such antibodies, equivalent binding molecules, such as antibody fragments (F(ab'), $F(ab')_2$, single chain antibodies, etc.), recombinant antibodies, chimeric antibodies, etc. may be employed. Such antibodies can be obtained using conventional methods with the gene products of either the BC534, BC538 or BC538.1 gene as an antigen. Such gene products are preferably obtained through the expression of the gene sequences described herein.

The simplest immunoassay involves merely incubating an anti-BC534, anti-BC538 or anti-BC538.1 antibody with a sample suspected to contain the target molecule -- the protein product of the BC534, BC538 or BC538.1 gene. The presence of the target molecule is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for example the sample can be passively bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting

the support to be in contact with a sample suspected to contain the target molecule. Target molecules that have become bound to the immobilized antibody can be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labelled, second antibody that is capable of binding to a second epitope of the target molecule. Immobilization of the labelled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labelled target. The presence of any target molecules in the sample competes with the labelled target molecules for antibody binding sites. Thus, the amount of labelled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

As indicated above, immunoassay formats may employ labelled antibodies to facilitate detection. Radioisotopic immunoassays ("RIAs") have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in Laboratory Techniques and Biochemistry in Molecular Biology, by Work, T.S., et al., North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein.

Enzyme-based immunoassay formats (ELISAs) have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such

that a large number of detection strategies -- colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in ELISA and Other Solid Phase Immunoassays (Kemeny, D.M. et al., Eds.), John Wiley & Sons, NY (1988), incorporated by reference herein. For these reasons, enzyme labels are particularly preferred.

No single enzyme is ideal for use as a label in every conceivable immunometric assay. Instead, one must determine which enzyme is suitable for a particular assay system. Criteria important for the choice of enzymes are turnover number of the pure enzyme (the number of substrate molecules converted to product per enzyme site per unit of time), purity of the enzyme preparation, sensitivity of detection of its product, ease and speed of detection of the enzyme reaction, absence of interfering factors or of enzyme-like activity in the test fluid, stability of the enzyme and its conjugate, availability and cost of the enzyme and its conjugate, and the like. Examples of suitable enzymes include peroxidase, acetylcholine esterase, alpha-glycerol phosphate dehydrogenase, alkaline phosphatase, asparaginase, β-galactosidase, catalase, delta-5-steroid isomerase, glucose oxidase, glucose-6-phosphate dehydrogenase, glucoamylase, glycoamylase, luciferase, malate dehydrogenase, peroxidase, ribonuclease, staphylococcal nuclease, triose phosphate isomerase, urease, yeast-alcohol dehydrogenase, etc. Peroxidase and urease are among the more preferred enzyme labels, particularly because of chromogenic pH indicators which make its activity readily visible to the naked eye.

In lieu of such enzyme labels, radioisotopic, chemiluminescent or fluorescent labels may be employed. Examples of suitable radioisotopic labels include ^{3}H,

^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, etc. Examples of suitable chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, an aequorin label, etc. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a fluorescamine label, etc.

As an alternative to such immunoassay formats, the presence of BC534, BC538 or BC538.1 in a cell can be determined by any means capable of detecting BC534, BC538 or BC538.1 mRNA. Thus, molecules comprising nucleic acid probes capable of hybridizing to the BC534 BC538 or BC538.1 sequences of Figure 1 may be used in the diagnosis of breast cancer. As used herein, a "probe" is a detectably labelled nucleic acid molecule that is capable of hybridizing to a defined site of a target molecule. Any of the nucleotide sequences disclosed herein can be used to define a probe; the general requirement for such use being merely that the nucleic acid molecule be sufficiently long (generally 10 or more nucleotides in length) that it possesses the capacity to form stable hybridization products with the target molecule. Any of a wide variety of labels (see above) may be used to label nucleic acids: enzyme labels (Kourilsky et al., U.S. Patent 4,581,333), radioisotopic labels (Falkow et al., U.S. Patent 4,358,535; Berninger, U.S. Patent 4,446,237), fluorescent labels (Albarella et al., EP 144914), chemical labels (Sheldon III et al., U.S. Patent 4,582,789; Albarella et al., U.S. Patent 4,563,417), modified bases (Miyoshi et al., EP 119448), etc.

Such nucleic acid based assays may use either DNA or RNA to detect the BC534, BC538 or BC538.1 mRNA. In one embodiment, the assays may be performed on RNA that has been extracted from breast cells. Alternatively, and more preferably, the assays may be done in situ on biopsied tissue.

Where the concentration of BC534, BC538 or BC538.1 mRNA in a sample is too low to be detected, such mRNA may be specifically amplified through the use of any of a variety of amplification protocols, such as PCR (Mullis, K.B., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Saiki, R.K., et al., Bio/Technology 3:1008-1012 (1985); Mullis K. et al., U.S. Patent 4,683,202; Erlich, H., U.S. Patent 4,582,788; Saiki, R. et al., US 4,683,194 and Mullis, K.B., et al., Met. Enzymol. 155:335-350 (1987), transcription-based amplification systems (Kwoh, D. et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173 (1989); Gingeras, T.R. et al., PCT appl. WO 88/10315 (priority: US Patent applications serial nos. 064,141 and 202,978); Davey, C. et al. (European Patent Application Publication no. 329,822), etc.

In yet another embodiment, the diagnosis of BC534, BC538, BC538 or BC53/reg expression is performed using a ribozyme produced from nucleic acid molecules having a sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

B. Prognostic Uses

The present invention additionally provides a capacity to predict whether an individual is at risk for breast cancer. Thus, any of the above-described assays may be performed on an asymptomatic individual in order to assess that individual's predisposition to breast cancer.

C. Therapeutic Uses

Significantly, the present invention provides a means for treating breast cancer. Such treatment may be either "prophylactic" or "therapeutic." A prophylactic treatment is one that is provided in advance of any symptom of breast cancer in order to prevent or attenuate any subsequent onset of the disease. A therapeutic treatment is one that is provided in response to the onset of a symptom of breast cancer, and serves to attenuate an actual symptom of the disease.

In one embodiment, such treatment is provided by administering to a patient in need of such treatment an effective amount of an antibody, or an antibody fragment (F(ab'), $F(ab')_2$, single chain antibodies, etc.) that is capable of binding to the product of the BC534, BC538 or BC 538.1 gene. As used herein, an effective amount is an amount sufficient to mediate a clinically significant change in the severity of a symptom, or a clinically significant delay in the onset of a symptom.

As will be appreciated, for acute administration, polyclonal or monoclonal antibodies (or fragments of either) may be administered. More preferably, and especially for chronic administration, the use of non-immunogenic antibodies is preferred. Such molecules can be pseudo-homologous (i.e. produced by a non-human species, but altered to a form that is immunologically indistinct from human antibodies). Examples of such pseudo-homologous molecules include "humanized" (i.e. non-immunogenic in a human) prepared by recombinant or other technology. Such antibodies are the equivalents of the monoclonal and polyclonal antibodies, but are less immunogenic, and are better tolerated by the patient.

Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e.

chimeric antibodies) (Robinson, R.R. et al., International Patent Publication PCT/US86/02269; Akira, K. et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison, S.L. et al., European Patent Application 173,494; Neuberger, M.S. et al., PCT Application WO 86/01533; Cabilly, S. et al., European Patent Application 125,023; Better, M. et al., Science 240:1041-1043 (1988); Liu, A.Y. et al., Proc. Natl. Acad. Sci. USA 84:3439-3443 (1987); Liu, A.Y. et al., J. Immunol. 139:3521-3526 (1987); Sun, L.K. et al., Proc. Natl. Acad. Sci. USA 84:214-218 (1987); Nishimura, Y. et al., Canc. Res. 47:999-1005 (1987); Wood, C.R. et al., Nature 314:446-449 (1985)); Shaw et al., J. Natl.Cancer Inst. 80:1553-1559 (1988); all of which references are incorporated herein by reference). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (Science, 229:1202-1207 (1985)) and by Oi, V.T. et al., BioTechniques 4:214 (1986); which references are incorporated herein by reference).

Suitable "humanized" antibodies can alternatively be produced by CDR or CEA substitution (Jones, P.T. et al., Nature 321:552-525 (1986); Verhoeyan et al., Science 239:1534 (1988); Beidler, C.B. et al., J. Immunol. 141:4053-4060 (1988); all of which references are incorporated herein by reference).

In another embodiment, the nucleic acid molecules of the present invention may be mutated and expressed in order to identify BC534, BC538 or BC538.1 mutant gene products that can complex with and significantly inactivate normal BC534, BC538 or BC538.1 gene products present in a tumor cell. In one sub-embodiment, such mutated protein molecules may be administered to a patient. Alternatively, nucleic acid expressing such molecules may be administered.

In yet another embodiment, "antisense" or "triplex" nucleic acid molecules may be used to provide the desired therapy. As used herein, an "antisense oligonucleotide" is a nucleic acid (either DNA or RNA) whose sequence is complementary to the sequence of at least part of the BC534, BC538, BC538.1 the protein-encoding sequences or the regulatory sequences in BC53/reg described herein; such that it is capable of binding to, or hybridizing with, an endogenous BC534, BC538 or BC538.1 mRNA molecule, and can thereby impair (i.e. attenuate or prevent) its the translation into BC534, BC538 or BC538.1 gene products. A "triplex" molecule is a nucleic acid molecule that is capable of binding to double-stranded DNA in a manner sufficient to impair its transcription.

To act as a triplex oligonucleotide, the nucleic acid molecule must be capable of binding to the BC534, BC538, or BC538 gene sequences of the double-stranded DNA genome in a manner sufficient to impair the transcription of either gene. Triplex oligonucleotides are disclosed by Hogan, U.S. Patent 5,176,996 and by Varma et al., U.S. Patent 5,175,266. To act as an antisense oligonucleotide, the nucleic acid molecule must be capable of binding to or hybridizing with that portion of the BC534, BC538 or BC538.1 mRNA molecule which mediates the translation of the target mRNA; or BC53/reg which programs transcription of their mRNAs. Antisense oligonucleotides are disclosed in European Patent Application Publication Nos. 263,740; 335,451; and 329,882, in U.S. Patent 5,097,617 and in PCT Publication No. WO90/00624, all of which references are incorporated herein by reference. Such a molecule can be of any length that is effective for this purpose. Preferably, the antisense oligonucleotide will be about 10-30 nucleotides in length, most preferably, about 15-24 nucleotides in length.

Thus, in one embodiment of this invention, an antisense oligonucleotide that is designed to specifically block translation of a BC534 or BC538 mRNA transcript can be used to impair the expression of the BC534 or BC538 genes in a cell, and thereby provide a treatment for breast cancer.

In general, the antisense oligomer is prepared in accordance with the nucleotide sequence of BC534 or BC538 as reported herein.

The sequence of the antisense oligonucleotide may contain one or more insertions, substitutions, or deletions of one or more nucleotides provided that the resulting oligonucleotide is capable of binding to or hybridizing with the above-described translation locus of either BC534, BC538 or BC538.1 mRNA.

Any means known in the art to synthesize the antisense oligonucleotides of the present invention may be used (Zamechik et al., Proc. Natl. Acad. Sci. (U.S.A.) 83:4143 (1986); Goodchild et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:5507 (1988); Wickstrom et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:1028; Holt, J.T. et al., Molec. Cell. Biol. 8:963 (1988); Gerwirtz, A.M. et al., Science 242:1303 (1988); Anfossi, G., et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:3379 (1989); Becker, D., et al., EMBO J. 8:3679 (1989); all of which references are incorporated herein by reference). Automated nucleic acid synthesizers may be employed for this purpose. In addition, desired nucleotides of any sequence can be obtained from any commercial supplier of such custom molecules.

Most preferably, the antisense oligonucleotides of the present invention may be prepared using solid phase "phosphoramidite synthesis." The synthesis is performed with the growing nucleotide chain attached to a solid support derivatized with the nucleotide which will be the 3'-hydroxyl end of the oligonucleotide. The method involves the cyclical synthesis of DNA using monomer

units whose 5'-hydroxyl group is blocked (preferably with a 5'-DMT (dimethoxytrityl) group), and whose amino groups are blocked with either a benzoyl group (for the amino groups of cytosine and adenosine) or an isobutyryl group (to protect guanosine). Methods for producing such derivatives are well known in the art.

IV. Administration of the Molecules of the Present Invention

The above-described therapeutic agents of the present invention can be formulated according to known methods used to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of such agents, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb the agents. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate the agents into particles of a polymeric

material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

In one embodiment of the present invention, nucleic acid molecule(s) comprising antisense or triplex molecules, or encoding mutated BC534, BC538 or BC538.1 gene products may be administered using viral or retroviral vectors in accordance with the methods of "gene therapy".

The principles of gene therapy are disclosed by Oldham, R.K. (In: Principles of Biotherapy, Raven Press, NY, 1987), and similar texts. Disclosures of the methods and uses for gene therapy are provided by Boggs, S.S. (Int. J. Cell Clon. 8:80-96 (1990)); Karson, E.M. (Biol. Reprod. 42:39-49 (1990)); Ledley, F.D., In: Biotechnology, A Comprehensive Treatise, volume 7B, Gene Technology, VCH Publishers, Inc. NY, pp 399-458 (1989)); all of which references are incorporated herein by reference.

Although, as indicated above, such gene therapy can be provided to a recipient in order to treat (i.e. suppress, or attenuate) an existing condition, the principles of the present invention can be used to provide a prophylactic gene therapy to individuals who, due to inherited genetic mutations, or somatic cell mutation, are predisposed to breast cancer.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

Synthesis and Expression of the BC534, BC538 Genes

The BC534 and BC538 genes were prepared by oligonucleotide synthesis using solid phase gene assembly. Successive oligonucleotides phosphorylated and added at molar excess were attached stepwise to a growing chain anchored to a solid phase support. The assembled oligonucleotides were ligated with T4 DNA ligase and detached from the support by cleaving at an added restriction enzyme site. The constructed genes were processed in the EXPRESS system (Invitrogen Corp.: pTrcHis Xpress-Prokaryotic Expression and Purification system) according to the manufacturer's instructions.

In this manner, two DNA molecules, encoding proteins of 21 and 44 amino acids, respectively, were constructed. These nucleic acid sequences of these molecules are 100% homologous to regions on the antisense strand of human p53 gene. The expression of these proteins is repressed by the normal wild type p53 gene healthy in women; however, mutations, inherited or acquired, in regions of the p53 gene which are crucial for maintaining repression could permit the expression of various levels of these two antisense proteins. The antisense proteins are potential intracellular activators of tumor causing and tumor associated proteins and of an inhibitor of 17ß hydroxy steroid interaction with its receptor. The antisense relationship with p53 gene and the functions of these proteins make them ideal candidates for causative factors of p53 related female breast cancer and some

other cancers which correlate with this disease in families carrying the mutated p53 gene. Since these proteins are not expressed (or expressed at insignificant levels) in normal humans they are excellent targets for developing humanized antibodies against to prevent and cure the disease.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: PREDDIE, RICK E.
BERGMANN, JOHANNA E.

(ii) TITLE OF INVENTION: AGENTS FOR THE PREVENTION AND TREATMENT OF BREAST CANCER

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: US
(F) ZIP: 20231

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 32,680
(C) REFERENCE/DOCKET NUMBER: 683-006

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(B) TELEFAX: (202) 857-0939

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: BR534

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCAGAATGC AACAAGCCCA GACGGAAACC GTAGCTGCCC TGGTAGGTTT TCTGGGAAGG 60

GACAGAAGAT GACAGGGG 78

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: BR534

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gln Glu Ala Gln Thr Glu Thr Val Ala Ala Leu Val
1 5 10

Gly Phe Leu Gly Arg Asp Arg Arg
15 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: BR538

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCCATGGACG AGAGGCGGAA GGCAGTCTGG CTGCCAATCC AGGGAAGCGT GTCACCGTCG 60

TGGAAAGCAC GCTCCCAGCC CGAACGCAAA GTGTCCCCGG AGCCCAGCAG CTACCTGCTC 120

CCTGGACGGT GGCTCTAGAC 140

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: BR538

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Val Thr Arg Lys Ala Val Trp Leu Pro Ile Gln
1 5 10

Gly Ser Val Ser Pro Ser Trp Lys Ala Arg Ser Gln Pro
15 20 25

Glu Arg Lys Val Ser Pro Glu Pro Ser Ser Tyr Leu Leu
30 35

Pro Gly Arg Trp Leu
40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES
(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(vii) IMMEDIATE SOURCE:
(B) CLONE: BR538.1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCC ATG GCA GTG ACC CGG AAG GCA GTC TGG CTG CTG CAA GAG
GAA AAG TGG GGA TCC AGC 60

ATG AGA CGC TTC CAA CCC TGG GTC ACC TGG GCC TGC AGA GGA
GGA GGC CCC TCC GGG AAC 120

ACC ATG CCA GTG TCT GAG ACA GCT CGG CTT CCT GTG GTG CAG
GAA AAG AAT GGC TGC TTC 180

ACA TTC TCT CTT CCA ATG TTT CAC CAC AAC CCA AGC ACT CCT
GCC CCA CCC CAC ACC AGC 240

CAT GCA CTT CTT TGA 252

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:

(B) CLONE: BR538.1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Val Thr Arg Lys Ala Val Trp Leu Leu Gln Glu
1 5 10

Glu Lys Trp Gly Ser Ser Met Arg Arg Phe Gln Pro Trp
15 20 25

Val Thr Trp Ala Cys Arg Gly Gly Gly Pro Ser Gly Asn
30 35

Thr Met Pro Val Ser Glu Thr Ala Arg Leu Pro Val Val
40 45 50

Gln Glu Lys Asn Gly Cys Phe Thr Phe Ser Leu Pro Met
55 60 65

Phe His His Asn Pro Ser Thr Pro Ala Pro Pro His Thr
70 75

Ser His Ala Leu Leu
80

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: BR53/reg

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAAGCCAAG GAATACACGT GGATGAAGAA AAAGAAAAGT TCTGCATCCC CAGGAGAGAT 60

GCTGAGGGTG TGATGGGATG GATAAAAGCC CAAATTCAAG GGGGGAATAT TCAACTTTGG 120

GACAGGAGTC AGAGATCACA CATTAAGTGG GTAAACTATA AAAAAACACT

WHAT IS CLAIMED IS:

1. A nucleic acid molecule, substantially free of natural contaminants, that encodes a protein selected from the group consisting of BC534, BC538 and BC538.1.

2. The nucleic acid molecule of claim 1 that encodes BC534.

3. The nucleic acid molecule of claim 2 wherein said sequence is SEQ ID NO:1.

4. The nucleic acid molecule of claim 1 that encodes BC538.

5. The nucleic acid molecule of claim 4 wherein said sequence is SEQ ID NO:3.

6. The nucleic acid molecule of claim 1 that encodes BC538.1

7. The nucleic acid of claim 6 wherein said sequence is SEQ ID NO:5.

8. A protein, substantially free of natural contaminants, selected from the group consisting of a BC534 gene product, a BC538 gene product and a BC538.1 gene product.

9. The protein of claim 8 wherein said protein is a BC534 gene product.

10 The protein of claim 8 wherein said protein has a sequence of SEQ ID NO:2.

11. The protein of claim 8, wherein said protein is a BC538 gene product.

12. The protein of claim 11, wherein said protein has a sequence of SEQ ID NO:4.

13. The protein of claim 8, wherein said protein is a BC538.1 gene product.

14. The protein of claim 13, wherein said protein has a sequence of SEQ ID NO:6.

15. A reagent capable of diagnosing the presence of a molecule selected from the group consisting of a BC534 gene sequence, a BC534 mRNA transcript, a BC534 gene product, a BC538 gene sequence, a BC538 mRNA transcript, a BC538 gene product, a BC538.1 gene sequence, a BC538.1 mRNA transcript nd a BC538 gene product.

16. The reagent of claim 15, wherein said reagent is a nucleic acid molecule.

17. The reagent of claim 16, wherein said reagent is a ribozyme produced from nucleic acid molecules having a sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

18. The reagent of claim 15, wherein said reagent is obtainable by mutating a nucleic acid molecule having a sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

19. The reagent of claim 15, wherein said reagent comprises a nucleic acid sequence that is complementary to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7

20. The reagent of claim 15 wherein said reagent is a protein.

21. The reagent of claim 20, wherein said protein is an antibody, or a fragment of an antibody.

22. The antibody or fragment of an antibody of claim 21, which is capable of binding to a BC534 gene product.

23. The antibody or fragment of an antibody of claim 21, which is capable of binding to a BC538 gene product.

24. The antibody or fragment of an antibody of claim 21, which is capable of binding to a BC538.1 gene product.

25. A method of treating breast cancer which comprises providing to an individual, in need of such treatment, an effective amount of an inhibitor of BC534, BC538, BC538.1 and BC53/reg.

26. The method of claim 25, wherein said inhibitor is a protein.

27. The method of claim 26, wherein said inhibitor is an antibody, or fragment thereof.

28. The method of claim 25, wherein said inhibitor is a nucleic acid molecule.

FIGURE 1

(A)

5'- CCC AGA ATG CAA CAA GCC CAG ACG GAA ACC GTA
GCT GCC CTG GTA GGT TTT CTG GGA AGG GAC AGA
AGA TGA CAG GGG -3'

(B)

M Q E A Q T E T V A A L V G F L G R D R R

(C)

5'- TCC ATG GAC GAG AGG CGG AAG GCA GTC TGG CTG
CCA ATC CAG GGA AGC GTG TCA CCG TCG TGG AAA
GCA CGC TCC CAG CCC GAA CGC AAA GTG TCC CCG
GAG CCC AGC AGC TAC CTG CTC CCT GGA CGG TGG
CTC TAG AC -3'

(D)

M A V T R K A V W L P I Q G S V S P S W K A
R S Q P E R K V S P E P S S Y L L P G R W L

FIGURE 1 (cont.)

(E)

5'- TCC ATG GCA GTG ACC CGG AAG GCA GTC TGG CTG CTG
CAA CAG GAA AAG TGG GGA TCC AGC ATG AGA CGC TTC
CAA CCC TGG GTC ACC TGG GCC TGC AGA GGA GGA GGC
CCC TCC GGG AAC ACC ATG CCA GTG TCT GAG ACA GTC
CGG CTT CCT GTG GTG CAG GAA AAG AAT GGC TGC TTC
ACA TTC TCT CTT CCA ATG TTT CAC CAC AAC CCA AGC
ACT CCT GCC CCA CCC CAC ACC AGC CAT GCA CTT CTT
TGA -3'

(F)

M A V T R K A V W L L Q E E K W G S S M R R F Q
P W V T W A C R G G G P S G N T M P V S E T A R
L P V V Q E K N G C F T F S L P M F H H N P S T
P A P P H T S H A L L

(G)

5'- AAAAGCCAAGGAATACACGTGGATGAAGAAAAAGAAAAGTTCTGCAT
CCCCAGGAGAGATGCTGAGGGTGTGATGGGATGGATAAAAGCCCAAA
TTCAAGGGGGGAATATTCAACTTTGGGACAGGAGTCAGAGATCACAC
ATTAAGTGGGTAAACTATAAAAAAACACT -3'

underline = antigenic epitope

FIGURE 2A

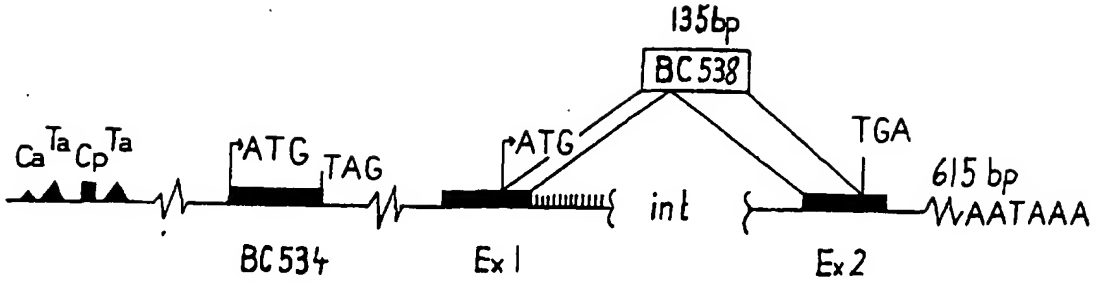
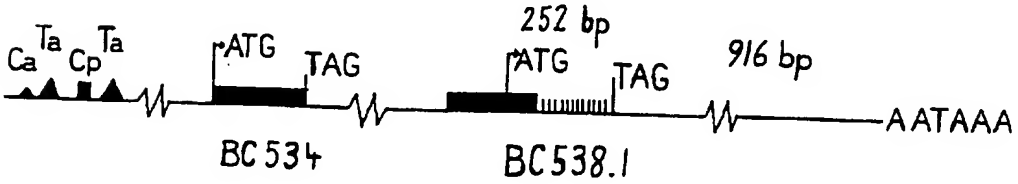


FIGURE 2B



Ca	=	CCAAT box
Ta	=	TATA box
Cp	=	Cap site
ATG	=	open reading frame
Ex	=	exon
int	=	intron
TAG, TGA	=	stop codon
AATAAA	=	transcription termination signal

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 94/00651

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 A61K37/02 C12P21/02 C07K13/00 C12N15/11
C12N9/00 C12P21/08 G01N33/53 A61K39/395 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C12P C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR AND CELLULAR BIOLOGY vol. 5, no. 7 , July 1985 , WASHINGTON US pages 1601 - 1610 HARLOW, E. ET AL. 'Molecular cloning and in vitro expression of a cDNA clone for human cellular tumor antigen p53' see figure 4 ---	1
A	NUCLEIC ACIDS RES 19 (6). 1991. 1338 BERGMANN J, ET AL. 'A PROTEIN DRP90 ENCODED ON THE LEFTWARDS STRAND OF SOYBEAN NODULE URATE OXIDASE cDNA BINDS TO A REGULATORY SEQUENCE IN LEGHEMOGLOBIN C-3 GENE.' see the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 1994	01. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,90 09180 (BOARD OF REGENTS,THE UNIVERSITY OF TEXAS SYSTEM) 23 August 1990 cited in the application see page 6, line 25 - page 7, line 5 see claim 8 ---	15,16, 25,28
T	NUCLEIC ACIDS RESEARCH vol. 22, no. 10 , May 1994 , ARLINGTON, VIRGINIA US pages 1903 - 1908 MERINO, E. ET AL. 'Antisense overlapping open reading frames in genes from bacteria to humans' -----	

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/EP 94/00651

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 25-28 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 94/00651

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9009180	23-08-90	US-A-	5087617	11-02-92
		AU-B-	635002	11-03-93
		AU-A-	5085690	05-09-90
		EP-A-	0458829	04-12-91
		JP-T-	4505752	08-10-92
		US-A-	5248671	28-09-93